

# Comparison of use rates and treatment timing with glyphosate to control Mexican water lily

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## ABSTRACT

Mexican water lily (*Nymphaea mexicana* Zuccarini) originates from Mexico and the southeastern United States. It has been widely distributed, and dense infestations restrict water movement, contribute to siltation, and decrease the economic utility, recreational, and environmental values of water bodies. Following the expansion of Mexican water lily in Victoria, Australia, and associated concerns about low dissolved oxygen, a control program using glyphosate (N-(phosphonomethyl)glycine) was implemented. The current research project was undertaken to determine (a) the efficacy of glyphosate application during spring and autumn compared with standard summer application, (b) the efficacy of a low application rate, and (c) the rate of dieback, and oxygen depletion potential, following each of the above scenarios, to develop a herbicide application strategy to minimize the potential for low dissolved oxygen levels and maintain control of Mexican water lily. The 2 experimental sites in the Goulburn Weir system had replicate treatment plots with two glyphosate rates (1.08 and 2.16 kg ai ha<sup>-1</sup> and untreated control plots) and applications at different months over 2 successive years. Time of application was the most significant factor in determining efficacy, with treatment during late summer/autumn resulting in the longest duration of low Mexican water lily cover. There were no differences in efficacy attributed to herbicide rate. Based on the results of this study, long-term low cover of Mexican water lily cannot be achieved with single annual applications (up to 2.16 kg ai ha<sup>-1</sup>). Therefore, alternative strategies need to be employed, such as applying glyphosate twice per year or using higher rates.

**Key words:** aquatic plant management, herbicide efficacy, *Nymphaea mexicana*, oxygen, seasonal treatment.

## INTRODUCTION

Mexican water lily (*Nymphaea mexicana* Zuccarini) is a yellow-flowered perennial aquatic plant that originates from Mexico and the southeastern United States. It is a

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parent of many hybrid cultivars of ornamental water lilies that have been distributed to many countries as pond plants. Naturalized plants in Australia and elsewhere outside its native range, including New Zealand, southern Africa, and Spain, are likely to be of hybrid origin but have characters diagnostic of the species (Queensland Government 2011). These include upright rhizomes (erroneously known as tubers), creeping fleshy stolons with attached “brood bodies” consisting of a number of buds and a bunch of short, yellow, banana-shaped roots, giving the plant its alternative common name of banana water lily (Rickettson 1995). Plants in Victoria (Australia) differ from this description in that rhizomes are horizontal and stolons are relatively short, producing smaller brood bodies lacking the banana-like roots. This indicates the plants are hybrids with other *Nymphaea* L. species. Further, they occasionally bears viviparous flowers (T. Dugdale, unpub. data). Regardless of their taxonomic status, these water lilies are highly invasive within the study area. Plants can rapidly colonize shallow (up to 2 m (6.5 feet) deep), nutrient-rich waters, and can result in heavy infestations in still-water lakes, reservoirs, ponds, and slow-moving channels (DiTomaso and Healy 2003). Heavy infestations can restrict water movement, contribute to siltation, reduce gas exchange between air and water, and decrease the recreational value of a water body (Capperino and Schneider 1985).

Mexican water lily (along with other aquatic weed species) was likely introduced into lakes and waterways of northern Victoria as a result of careless disposal of aquarium plants (G-MW 2009). It is presently in Lake Nagambie, Goulburn Weir Pool, Gunbower Creek, and Broken River, as well as in Lake Benalla, and continues to spread (G-MW 2009).

Goulburn-Murray Water (G-MW) manages the major dams, irrigation supply, and drainage systems, surface-water diversions, and groundwater in the Goulburn River catchment, including rivers and streams that are natural carriers, as well as Goulburn Weir pool (and associated backwaters) that were formed upstream of the Goulburn weir (constructed in 1891). These lakes and waterways have multiple uses and many stakeholders, with stored water used for crop irrigation, stock and domestic water supply, and recreation, as well as the recognized biodiversity values of the system.

Within the G-MW-managed water bodies, Mexican water lily has a negative impact on both water quality and amenity values of these systems (G-MW 2009), such as reduced habitat value for fauna and flora, restricted water exchange between backwater areas and river channels, impeded access, and reduced aesthetic values, including foul odor associated with declining water quality (Finlay et al. 2008).

In response, G-MW implemented a spray program with glyphosate (N-(phosphonomethyl)glycine) over a 7-yr period (1996 to 2003) during which the area of Mexican water lily within the weir pool was reduced from 200 to ca. 30 ha (494 to 70 ac) (Finlay et al. 2008, G-MW 2009). Water Eco Science study found no direct impact of the spray program on water quality (Francis and Crapper 2004). However, the program was suspended from 2004 to 2008 following fish deaths in the Goulburn River downstream of the weir. It was suspected that water with depleted oxygen levels passed over the weir and caused the fish deaths downstream, but an audit by Environmental Protection Authority Victoria was unable to establish a cause.

Although it is recognized that rapid plant dieback and decay can result in very low oxygen levels in the water, with associated potential for nutrient pulses and fauna death, G-MW has responded to complaints from stakeholders about poor water quality following expansion of Mexican water lily in the system and currently controls Mexican water lily in water bodies they manage with glyphosate, under a strict risk management framework (Finlay et al. 2008).

Mexican water lily can be effectively controlled with glyphosate at  $6 \text{ kg ai ha}^{-1}$  ( $5.35 \text{ lb ai ac}^{-1}$ ; Champion 1999, 2003, 2007); however, this rate exceeds that which is allowed by regulatory authorities. Although rapid die-off occurs at lower rates, the duration of this control has not been established. The current research project was undertaken to determine efficacy of glyphosate at low rates and to determine if herbicide could be applied at lower rates or cooler times of the year to minimize deoxygenation of the water, without compromising efficacy of control. Application at lower rates might result in a slower decomposition process and therefore maintenance of higher oxygen levels in the water column. The oxygen holding capacity of the water is greater in cool water, so application at cooler times of year may also result in greater oxygen level in the water.

The objectives were to determine (a) the efficacy of application during spring and autumn compared with standard summer application, (b) the efficacy of a low application rate compared to the label rate, and (c) the rate of dieback, and therefore oxygen depletion potential, following each of the above scenarios. The purpose then was to use the research data to develop a herbicide application strategy to minimize low oxygen levels in the water and maintain satisfactory control of Mexican water lily.

## MATERIALS AND METHODS

A replicated and randomized trial was set up in the summer of 2008 and 2009 that included 2 different experimental sites (Basleys' backwater and Picnic Point) within the Goulburn Weir Pool backwater (Figure 1), with 2 herbicide rates and applications at different times (months) over 2 successive years. Both locations had shallow water with little or no water flow and were characterized by 100% surface cover of Mexican water lily, with few other aquatic plant species present (e.g., the submerged *Cabomba caroliniana* Gray).



Figure 1. Location of the 2 herbicide trial sites, Basleys and Picnic Point ( $36.747791^{\circ}\text{S}$ ;  $145.136175^{\circ}\text{E}$ ), in the backwater between Goulburn Weir pool and Lake Nagambie, Victoria, Australia.

At each location 60 adjacent treatment plots (5 by 4 m) were marked out with stakes that emerged above the water. There were 3 replicate plots for each herbicide treatment ( $3 \text{ L ha}^{-1}$  or  $6 \text{ L ha}^{-1}$  ( $1.29$  or  $2.57$  quarts  $\text{ac}^{-1}$ )) glyphosate, i.e.,  $1.08$  and  $2.16 \text{ kg ai ha}^{-1}$  of Weedmaster Duo<sup>®</sup>1,  $360 \text{ g ai L}^{-1}$  (36%) for each treatment month, and 3 untreated control plots at each of the 2 locations. Herbicide application was monthly from spring (November) through to early winter (June to July) in the 1st year of treatment. In the 2nd year herbicide was reapplied to plots at the same rates, and month of treatment from November through to July at Basleys' backwater and through to June at Picnic Point. Treatment plots were sprayed with glyphosate from a spray boat using calibrated equipment with a foam jet nozzle (0010) to minimize drift. Spray volume was  $600 \text{ L ha}^{-1}$ . A total of 9 applications per treatment were undertaken and monitored at least monthly. Monitoring involved a visual assessment of percent cover of Mexican water lily above the water by 2 assessors. A 0.5-m band around the perimeter of each plot was ignored when estimating cover to negate the impact of leaves originating from adjacent plots. During application and monitoring events, water temperature and dissolved oxygen (DO) were recorded midmorning, ca. 30 cm below the water surface in

one of the replicate plots to be treated that day with an InsiteIG 310<sup>®</sup> optical oxygen meter<sup>2</sup>, and water depth was recorded, along with comments on general plant health (leaf size and condition), ambient temperature, and weather conditions. To provide an indication of the background DO dynamics, DO was measured 10 cm above the substrate in an area adjacent to the plots at Picnic Point, under Mexican water lily with 100% cover during an 8-wk period in summer 2010. The probe was a Greenspan<sup>®</sup>ODO3000<sup>3</sup> fitted with an optical DO sensor, set to record and log at 30-min intervals.

### Statistical analysis

Visual assessments of percent cover of Mexican water lily were used to estimate the duration of low water lily cover following herbicide application and the time to low cover. The duration of low cover was defined as the total number of months with  $\leq 20\%$  water lily cover during the period of 1 yr following herbicide application. Time to low cover was then determined as the time interval (weeks) between herbicide application and the reduction in water lily cover to  $\leq 20\%$ . The duration of low cover and time to low cover were estimated for each month of herbicide application (November–August), herbicide rate (control, 3 L ha<sup>-1</sup>, or 6 L ha<sup>-1</sup>) and location (Basleys or Picnic Point). Time to low cover was stratified by the frequency of assessment (2 or 3 mo<sup>-1</sup>) as the frequency of assessment affected the precision of estimation. Months of herbicide application with fewer than 2 assessments per month (May, June, and July in Year 1 and all of Year 2) were excluded.

*t* tests were used for binary comparisons of outcome measures between herbicide rates and locations. Ordinary least squares (OLS) multiple regression analysis was used to model nonlinear and multivariate relationships. *F* tests were used to assess the contribution of individual independent variables to the OLS models. Stata<sup>®</sup>/SE 12.1<sup>4</sup> was used for all statistical analyses.

## RESULTS AND DISCUSSION

Mexican water lily showed clear seasonal trends with winter dieback of mature plants, new growth in spring, and increased cover over summer months within the control plots (Figure 2). This resulted in rapid increase in plant cover to ca. 100% by midspring (early November). At monthly site visits midmorning water temperatures ranged from 9 to 25 C, DO fluctuated from 4.8 mg L<sup>-1</sup> (January) to 10 mg L<sup>-1</sup> (June and July), and water depth varied between 0.3 and 1.2 m (all data from one of the replicate plots immediately prior to herbicide application). During January and February 2010 in the reference area, average daily DO ranged between 0 and 6 mg L<sup>-1</sup> and daily DO fluctuations were large (Figure 3).

Herbicide treatments were efficacious and did not differ between the 2 treatment rates (Tables 1 and 2), with both 3 and 6 L ha<sup>-1</sup> (1.08 or 2.16 kg ai ha<sup>-1</sup>) treatments resulting in a dramatic reduction in the cover of Mexican water lily a month from application, for all months in which the herbicide was applied (Figure 2).

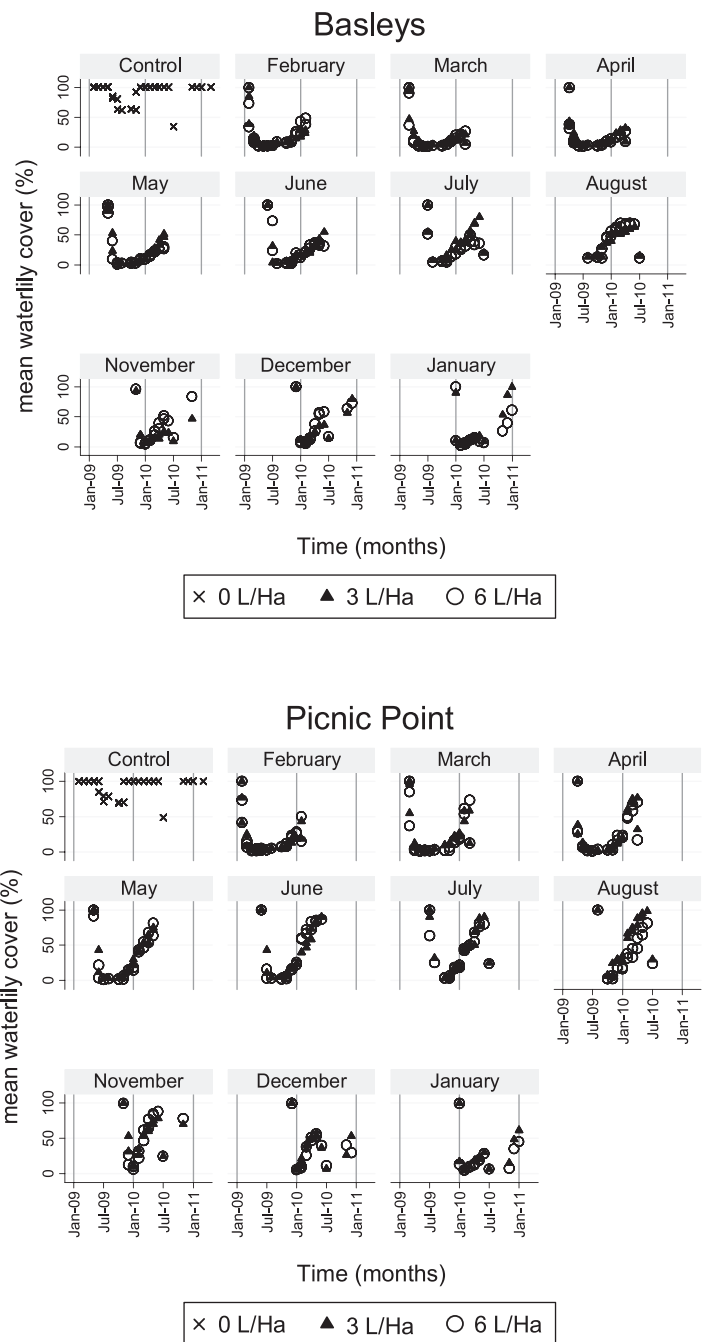


Figure 2. Mexican water lily response to herbicide application at different months (November to August), treatment rates, and plot location (Basleys or Picnic Point). Each estimate represents the mean cover (%) of 3 replicate measures, and is shown for the 12 mo following the 1st herbicide application and the duration of the study for treatment and control plots, respectively. The month of herbicide application is indicated above each graph and the vertical lines represent 1-yr intervals.

Dieback of aquatic plants is associated with a drop in DO as the plant decays, which is most rapid in the first few days after plant death (Carpenter and Greenlee 1981). It is difficult to predict DO depletion after herbicide application because several factors and their interactions must be considered (water temperature, flushing rate, depth, mac-

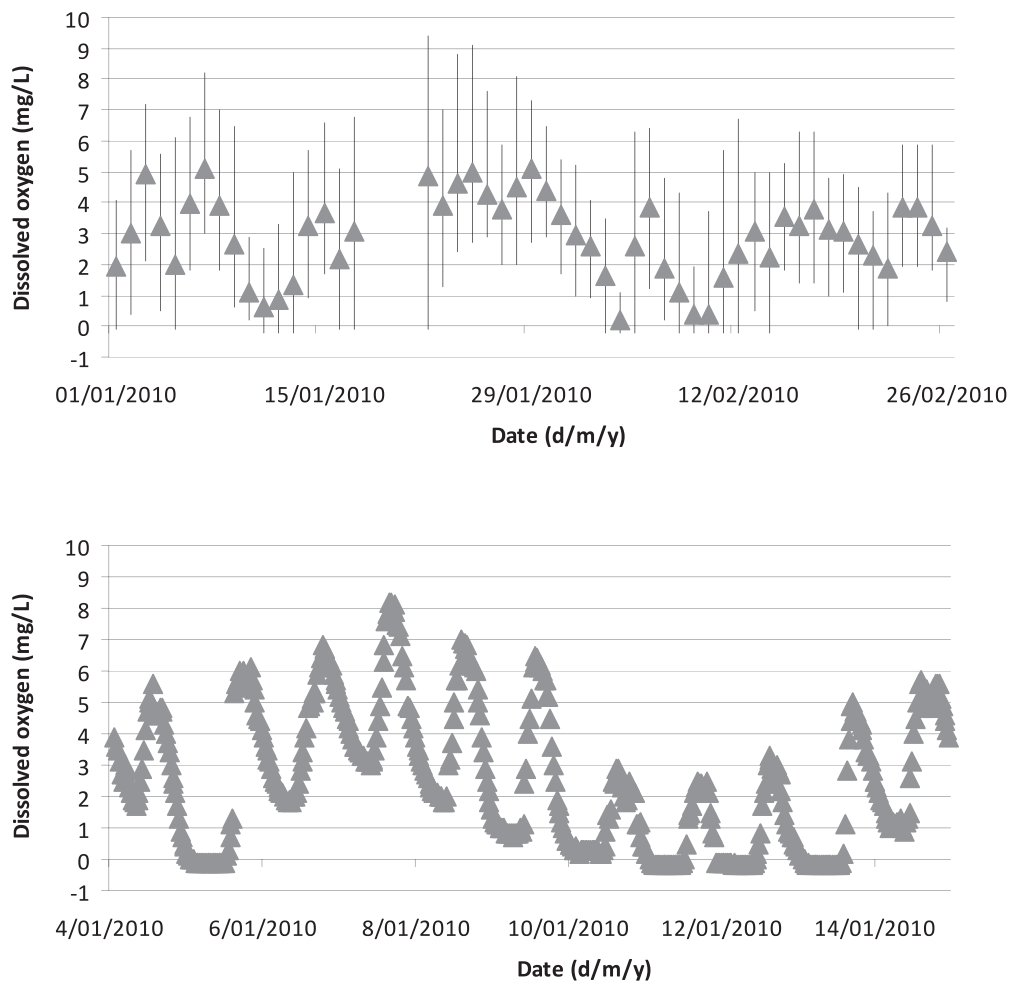


Figure 3. Dissolved oxygen (DO) at daily (top) and 30-min intervals (bottom) from 10 cm above the sediment in a reference area at Picnic Point. Daily values shown are maximum and minimum, with average DO concentration indicated by the triangle.

rophyte biomass, macrophyte nitrogen concentration), none of which in isolation have an overriding influence (Carpenter and Greenlee 1981). However, where dieback is rapid a more intense local DO demand could be expected than where it is slow. Hence, the rate of Mexican water lily dieback was used as a theoretical indicator of local DO

demand, and therefore potential for low water column DO. Although cover appeared to decline more quickly when treated at 6 L ha<sup>-1</sup> than at 3 L ha<sup>-1</sup>, there was no difference detected in the speed of dieback with application rate, location, or application month for the February to April period (Tables 3 and 4), as such associated differences in DO

TABLE 1. DURATION OF LOW MEXICAN WATER LILY COVER ( $\leq 20\%$ ) OVER A 12-MO PERIOD FOLLOWING THE 1ST HERBICIDE APPLICATION. OBSERVED MEAN ESTIMATES ( $\pm$  STANDARD DEVIATION) FOR EACH LOCATION (BASLEYS AND PICNIC POINT) AND TREATMENT (CONTROL, 3 AND 6 L ha<sup>-1</sup>) ARE SHOWN. THE DURATION OF LOW COVER WAS MEASURED IN MONTHS.

Month of Application	Control <sup>1</sup>		3 L ha <sup>-1</sup>		6 L ha <sup>-1</sup>	
	Basleys	Picnic Point	Basleys	Picnic Point	Basleys	Picnic Point
November	0.3 $\pm$ 0.5	0	8.3 $\pm$ 3.5	1.7 $\pm$ 1.2	5.0 $\pm$ 0	3.0 $\pm$ 1.0
December	0.3 $\pm$ 0.5	0	6.3 $\pm$ 4.0	7.0 $\pm$ 2.6	5.7 $\pm$ 3.8	6.3 $\pm$ 2.5
January	0.3 $\pm$ 0.5	0	5.7 $\pm$ 0.6	7.3 $\pm$ 2.9	9.0 $\pm$ 3.5	9.7 $\pm$ 1.2
February	0	0	11.0 $\pm$ 1.7	10.3 $\pm$ 1.5	10.0 $\pm$ 1.0	10.0 $\pm$ 0
March	0	0	11.0 $\pm$ 1.7	9.3 $\pm$ 0.6	11.3 $\pm$ 0.6	9.7 $\pm$ 0.6
April	0	0	9.7 $\pm$ 2.1	8.7 $\pm$ 0.6	11.3 $\pm$ 0.6	8.7 $\pm$ 0.6
May	0	0	8.7 $\pm$ 0.6	6.7 $\pm$ 0.6	9.3 $\pm$ 1.5	7.7 $\pm$ 0.6
June	0	0	7.7 $\pm$ 0.6	5.3 $\pm$ 0.6	7.3 $\pm$ 1.5	6.0 $\pm$ 1.7
July	0.3 $\pm$ 0.5	0	6.7 $\pm$ 1.5	5.0 $\pm$ 1.7	7.7 $\pm$ 0.6	5.3 $\pm$ 1.5
August	—	0	—	—	—	—

<sup>1</sup>n = 6 location<sup>-1</sup>.

TABLE 2. ORDINARY LEAST SQUARES MULTIPLE REGRESSION MODEL OF LOW MEXICAN WATER LILY COVER DURATION WITHIN 1 YR SINCE THE 1ST HERBICIDE APPLICATION, WITH PREDICTED ESTIMATES OF LOW COVER DURATION.

Independent Variables	Predicted Duration (mo) (95 % Confidence Interval)	$\Delta$ Duration ( <i>P</i> -value)	$\Delta R^{21}$	<i>F</i> -test ( <i>P</i> -value)
<b>Rate</b>				
3 L ha <sup>-1</sup>	7.5 (6.9–8.1)	<sup>2</sup>	0.005	0.323
6 L ha <sup>-1</sup>	7.9 (7.4–8.3)	0.37 (0.323)		
<b>Place</b>				
Picnic Point	8.3 (7.7–8.9)	<sup>2</sup>	0.047	0.002
Basleys	7.1 (6.7–7.5)	1.19 (0.002)		
<b>Month of application</b>				
November	4.2 (3.0–5.4)		0.473	<0.001
December	6.6 (5.8–7.4)			
January	8.3 (7.7–8.8)			
February	9.4 (8.9–9.8)			
March	9.8 (9.3–10.3)	<sup>3</sup> (<0.001)		
April	9.6 (9.2–10.0)			
May	8.8 (8.4–9.2)			
June	7.3 (6.9–7.7)			
July	5.2 (4.6–5.9)			
Model total	—	—	0.524	<0.001

<sup>1</sup>Change in  $R^2$  after the addition of the indicated independent variable to the model.

<sup>2</sup>Reference group.

<sup>3</sup>Variable change in Duration due to a quadratic relationship between Duration and the Time of application (see Figure 4).

are not expected. However, there was a difference in the speed of dieback for the application months November to January (Table 4). Plots treated in January died back to  $\leq 20\%$  cover in 2.3 wk compared to ca. 4 wk for all other months (Table 4), for reasons that are not apparent.

What was different between the treatment months was the duration that the Mexican water lily cover remained low (at 20% cover or less). The duration of low cover was strongly associated with location (Basleys compared with Picnic Point,  $P = 0.002$ ) and the month of herbicide application ( $P < 0.001$ ) (Table 2). The month of application was the strongest predictor; it accounted for 47% of the variability in the duration of low cover (Table 2). From the model, estimates of low cover duration were predicted (Table 2) and illustrate that late-summer to early-autumn herbicide application provides the longest period of low water lily cover (Figure 4).

For the treatments in the present study, Mexican water lily always grew back to problematic levels within 12 mo. There are at least 2 possible explanations for the differences observed in duration of low cover, which are longest for applications made from midsummer to autumn. The 1st explanation is that the longest duration of low cover was achieved at that time of year because the herbicide was translocated down into the rhizomes more effectively, thus having greater phytotoxicity. This is a common observation and is usually attributed to the net downward flow of solutes through the phloem at that time of year, taking the herbicide with it. A 2nd explanation is that Mexican water lily exhibits a strong seasonal growth trigger, whereby growth is triggered by conditions that occur only in spring (October to December). For example, for plants at both Basleys' backwater and Picnic Point, increase in cover began in November in the control plots and the plots treated from February through August. For the February treatment plots,

TABLE 3. COMPARISON BETWEEN HERBICIDE RATES (3 AND 6 L HA<sup>-1</sup>) AND THE TIME TAKEN TO ACHIEVE LOW MEXICAN WATER LILY COVER FOLLOWING THE 1ST HERBICIDE APPLICATION. MEAN ESTIMATES FOR WEEKLY PERIODS ( $\pm$  STANDARD DEVIATION [SD]) OF 3 REPLICATES ARE SHOWN FOR BOTH LOCATIONS, BASLEYS AND PICNIC POINT.

Assessments per Month	Month of Herbicide Application	Picnic Point		Basleys	
		3 L ha <sup>-1</sup> (wk $\pm$ SD)	6 L ha <sup>-1</sup> (wk $\pm$ SD)	3 L ha <sup>-1</sup> (wk $\pm$ SD)	6 L ha <sup>-1</sup> (wk $\pm$ SD)
3	February	4.3 $\pm$ 2.1	4.0 $\pm$ 0	4.0 $\pm$ 2.0	4.0 $\pm$ 2
	March	4.0 $\pm$ 0	4.0 $\pm$ 0	5.0 $\pm$ 3.6	3.3 $\pm$ 1.2
	April	4.0 $\pm$ 1.7	4.0 $\pm$ 1.7	5.3 $\pm$ 0.6	4.0 $\pm$ 1.7
	Average (wk)	4.1 $\pm$ 1.4	4.0 $\pm$ 0.9	4.8 $\pm$ 2.2	3.8 $\pm$ 1.5
	<i>t</i> test ( <i>P</i> value)		0.839		0.27
2	November	6.0 $\pm$ 1.7	3.3 $\pm$ 0.6	4.3 $\pm$ 2.3	3.0 $\pm$ 0
	December	4.0 $\pm$ 0	4.0 $\pm$ 0	4.0 $\pm$ 0	4.0 $\pm$ 0
	January	2.7 $\pm$ 1.2	2.7 $\pm$ 1.2	2.0 $\pm$ 0	2.0 $\pm$ 0
	Average (wk)	4.2 $\pm$ 1.8	3.3 $\pm$ 0.9	3.4 $\pm$ 1.6	3.0 $\pm$ 0.9
	<i>t</i> test ( <i>P</i> value)		0.198		0.472

TABLE 4. ORDINARY LEAST SQUARES MULTIPLE REGRESSION MODEL OF THE TIME TAKEN TO ACHIEVE LOW MEXICAN WATER LILY COVER FOLLOWING THE 1ST HERBICIDE APPLICATION.

Models (Time Restrictions)	Independent Variables	$\Delta$ Time-to-low Cover (wk)			Predicted Time (wk)	$\Delta R^{21}$	F-test (P-value)
		$\Delta$ (wk)	P-value	95% Confidence Interval			
February–April	<b>Rate</b>						
	3 L ha <sup>-1</sup>	2	2	2	4.4	0.034	0.299
	6 L ha <sup>-1</sup>	-0.6	0.299	-1.6 to 0.5	3.9		
	<b>Location</b>						
	Basleys	2	2	2	4.3	0.006	0.676
	Picnic Point	-0.2	0.676	-1.3 to 0.9	4.1		
	<b>Month</b>						
	February	2	2	2	4.1	0.006	0.896
	March	~0	<1.00	-1.4 to 1.4	4.1		
	April	0.3	0.685	-1.0 to 1.5	4.3		
Constant	4.47	<0.001	3.1 to 5.8	3			
Model total		3	3	3	0.046	0.575	
November–January	<b>Rate</b>						
	3 L ha <sup>-1</sup>	2	2	2	3.8	0.062	0.064
	6 L ha <sup>-1</sup>	-0.7	0.064	-1.4 to 0.4	3.2		
	<b>Location</b>						
	Basleys	2	2	2	3.2	0.043	0.119
	Picnic Point	0.6	0.119	-0.2 to 1.3	3.8		
	<b>Month</b>						
	November	2	2	2	4.2	0.38	<0.001
	December	-0.2	0.723	-1.1 to 0.8	4		
	January	-1.8	0.001	-2.9 to -0.8	2.3		
Constant	2.4	<0.001	1.8 to 3.0	3			
Model total		3	3	3	0.484	<0.0001	

<sup>1</sup>Change in R<sup>2</sup> after the addition of the indicated independent variable to the model.

<sup>2</sup>Reference group.

<sup>3</sup>Not applicable.

this represents a period of 9 mo that the rhizomes were dormant.

Glyphosate was shown to be a promising herbicide for water lily (both *Nymphaea* and the related *Nuphar* Sm. species) control by several early English and U.S. studies

(Barrett 1974, Riemer and Welker 1974, Welker and Riemer 1983) with total control of *Nuphar lutea* (L.) Sibth. & Sm., *Nuphar lutea* subsp. *variegata* (Durand) E. O. Beal, and *Nymphaea odorata* Aiton, obtained when glyphosate was applied at rates of around 2 kg ai ha<sup>-1</sup>. Herbicide efficacy

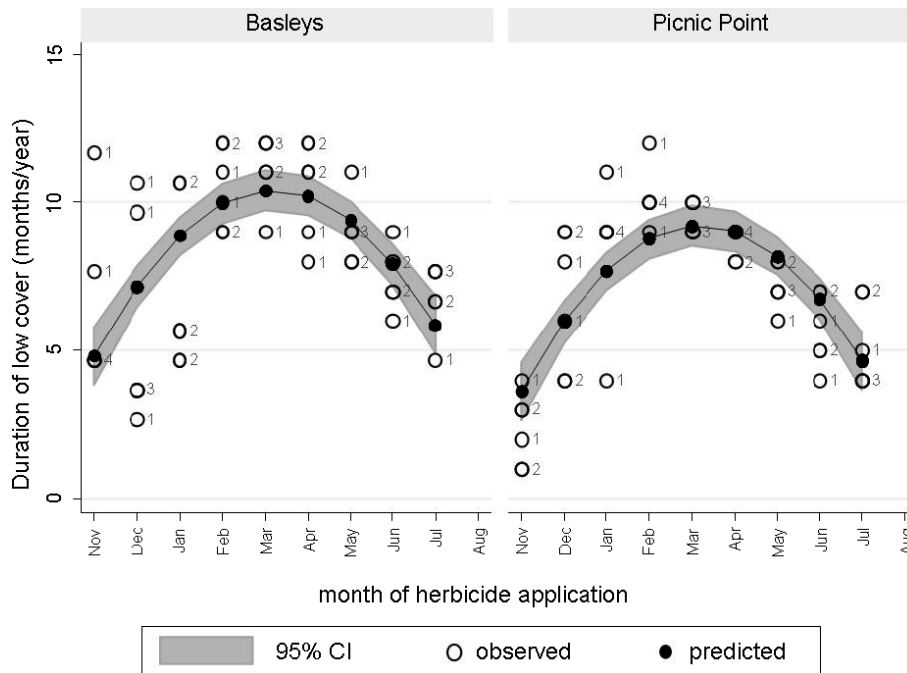


Figure 4. Illustration of the ordinary least squares regression model fit for duration of low Mexican water lily cover. The numbers beside each data point refer to the number of observations per data point.

on *Nymphaea odorata* was evident 1 mo after treatment (Riemer and Welker 1974), which is consistent with the findings of the present study. The New Zealand program to eradicate *Nuphar lutea* used aerial application of glyphosate at 6 kg ai ha<sup>-1</sup> (Champion 1991), with 2 applications over 2 yr reducing floating leaf canopy from 100% pretreatment to <5%. Lower application rates were used in a previous trial but led to short-term defoliation of the water lily with dense regrowth the following summer. Continued follow-up spot-spray treatment (annually) of seedling and rhizome regrowth using 3% glyphosate (360 g ai L<sup>-1</sup>) has now eradicated this species from the site (P. Champion, unpub. data). High rates of glyphosate have subsequently been used to control infestations of Mexican water lily at 2 New Zealand water bodies (Lake Rotokao, Hamilton, Waikato; and Waitakere Wetland, Bethells Beach, Auckland). In both cases treatments were made twice a year (early summer and autumn) spot-spraying floating leaves. Eradication was achieved at Lake Rotokao, and at Waitakere Wetland there was a reduction in area of water lily from 2 ha to 20 m<sup>2</sup>, both after 3 yr of treatment (Champion 1999, 2003, 2007; Dugdale and Reeves 2003). In all cases rhizome death was apparent with masses of floating rhizomes resulting from initial treatment. At Lake Rotokao, situated in an urban setting, dead rhizomes were mechanically harvested following initial herbicide treatment to reduce potential odor and associated decline in DO during decomposition (G. Angell, Aqua-Ag NZ, pers. comm.).

DO levels under areas of Mexican water lily in the Goulburn Weir pool that were not treated were severely depressed during warm months, with frequent periods of diurnal hypoxia (no data are available at other times; Figure 3). The low DO was probably due to a combination of factors reported to occur under heavy mats of floating vegetation, including natural turnover of standing crop, restricted water movement and mixing by dense floating canopies, and reduced oxygen holding capacity of warmer water (Sculthorpe 1967, Carpenter and Lodge 1986, Cronk and Fennessy 2001). Because of this, it could be argued that spraying Mexican water lily at this time will not result in a substantive further reduction, as levels are already hypoxic for much of the time. However, loading the water column with additional decaying plant material from herbicide-treated Mexican water lily may increase the duration and frequency of periods of diurnal hypoxia. Therefore, it is prudent from a risk management perspective to enact a strategy to reduce DO demand during this critical period. One such strategy is to reduce the cover of water lily in the warmer months of January through March, which has the potential to increase the surface area for gas exchange across the atmosphere–water interface and in turn increase DO levels (Sculthorpe 1967, Smart et al. 2009) during this period. However, in the current study, low cover of Mexican water lily in the warmest months (January through March) was only achieved with late-spring to early-summer (November and December) herbicide applications (Figure 2), which provided 4.2 mo (confidence interval [CI] 3.0 to 5.4) and 6.6 mo (CI 5.8 to 7.4) of low cover, respectively (Table 2). Although this is enough to get through this critical summer period, it would also add organic matter (and thus oxygen

demand) during this period of low ambient DO. Furthermore, Mexican water lily cover would be high by the following spring, meaning herbicide applications would be required on an ongoing basis.

Because none of the treatments tested here provided both low Mexican water lily cover in summer and low cover for a long duration, 2 alternative strategies are suggested. The 1st strategy is 2 herbicide applications annually, one in spring to early summer (e.g., November, December) to provide low lily cover in summer (January, February), the 2nd as temperatures start to cool in autumn (March, April) to achieve a longer duration of low cover. The 2nd strategy is to apply glyphosate at higher rates (6 kg ai ha<sup>-1</sup> vs. 2.2 kg ai ha<sup>-1</sup>), with or without a 2nd application in the same year. Although results in the present study do not demonstrate a change in control between the experimental rates, previous studies on Mexican water lily (Champion 1999, 2003, 2007) have shown that higher rates (e.g., 6 kg ai ha<sup>-1</sup>) may provide better control and significantly reduce the underwater biomass of rhizomes and stolons. It is likely that over successive years the rhizomes will be killed, resulting in substantially less Mexican water lily (as demonstrated by Champion 1999, 2003, 2007; Dugdale and Reeves 2003) and a corresponding decrease in the requirement for herbicide. Residue testing from the Goulburn Weir pool during Mexican water lily control in 1997 to 1998 showed that where glyphosate was applied at 2.16 kg ai ha<sup>-1</sup>, maximum glyphosate concentration in the water in the spray zone was 0.39 mg L<sup>-1</sup> while downstream glyphosate levels were <0.038 mg L<sup>-1</sup> (Francis and Crapper 2004). This is well below the maximum concentration of 1.2 mg L<sup>-1</sup> where 95% protection of freshwater species would occur (ANZECC 2000). It is therefore unlikely that a 3-fold increase in glyphosate application would breach these guidelines. Despite this, because we expect a higher rate of glyphosate will result in greater mortality of rhizomes (Champion 1999, 2003, 2007), which upon decay will create a larger DO demand than the current control program, it is suggested that higher rates are restricted to discrete blocks of Mexican water lily, adding new blocks to the treatment program each year in order to localize extreme DO depression events resulting from control. However, the effectiveness of this strategy would lead to reduced herbicide use following the initial control season, with corresponding decrease in area impacted by Mexican water lily and consequent improvement in DO. Harvesting of dead rhizomes needs to be considered and a follow-up program to control regrowth from viable rhizome fragments, brood bodies, or seedlings needs to be instigated.

In summary, the present study showed time (month) of application was the most significant factor in determining efficacy as measured by duration of low cover. Although there were some differences between the 2 experimental sites, data from both show that treatment during late summer to autumn (February to May) will yield >8.5 mo of low Mexican water lily cover. The study also showed there were no differences in efficacy or speed of dieback attributed to treatment rate <2.16 kg ai ha<sup>-1</sup>; therefore, an opportunity exists to reduce both the environmental load and economic costs associated with the use of the

herbicide. However, at either treatment rate, there would be a continued need for annual herbicide application. Therefore, alternative control strategies of either applying glyphosate twice a year or applying glyphosate at a higher rate are proposed. The former option will present a lower biomass of Mexican water lily at each application, resulting in a lower potential oxygen demand as it decays. The 2nd option is likely to reduce Mexican water lily abundance in the long term, leading to less use of herbicide.

## SOURCES OF MATERIALS

<sup>1</sup>Weedmaster® Duo. Nufarm Australia Limited, 103-105 Pipe Road, Laverton North, Victoria 3026, Australia.

<sup>2</sup>InsiteIG 310®. 80 Whisperwood Boulevard, Suite 107, Slidell, LA 70458, USA.

<sup>3</sup>Greenspan® ODO3000, Goyon Controls Company Propriety Limited, 268 Milperra Road, Milperra, New South Wales 2214, Australia

<sup>4</sup>Stata®/SE 12.1, StataCorp LP, 4905 Lakeway Drive, College Station, Texas 77845-4512, USA.

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